PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 12 September 2000 (12.09.00)	
International application No. PCT/US99/30338	**Applicant's or agent's file reference** BB1324 PCT1
International filing date (day/month/year) 20 December 1999 (20.12.99)	**Priority date** (day/month/year) 21 December 1998 (21.12.98)
Applicant FAMODU, Omolayo, O. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

10 July 2000 (10.07.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Henrik Nyberg
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PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
BB1324 PCT1		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/30338	20/12/1999	21/12/1998
International Patent Classification (IPC) or national classification and IPC C12N15/54		
Applicant E.I. DU PONT DE NEMOURS AND COMPANY et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☒ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
10/07/2000	23.03.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Mundel, C Telephone No. +49 89 2399 7314

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US99/30338

I. Basis f th report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-24 as originally filed

Claims, No.:

1-23 as originally filed

Sequence listing part of the description, pages:

1-9, filed with the letter of 07.02.00

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

   ☐ copy of the earlier application whose priority has been claimed.

   ☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

   Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:
   see separate sheet

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-6, 9-17 and 19-23
	No:	Claims	7, 8 and 18
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-23
Industrial applicability (IA)	Yes:	Claims	1-23
	No:	Claims	

2. Citations and explanations
   see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item II
Priority

The attention of the applicant is drawn to the fact that all the sequences cited in the present application can not claim the priority date of 21.12.98. Only the sequences set forth in SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:9 and SEQ ID NO:10 have been disclosed in the priority document. Thus, all the documents cited P, X in the International Search Report are considered as relevant for the evaluation of the novelty and inventive step of the claims as far as the sequences set forth in SEQ ID NO:1 to 4 and 7 and 8 are concerned.

Re Item V
Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The present application refers to nucleic acid sequences encoding putative soybean isoflavone-O-methyltransferase and to the corresponding polypeptides and to compositions comprising said nucleic acid sequences or polypeptides. The application also refers to chimeric genes comprising said nucleic acid sequences operably linked to suitable regulatory sequences, to host cells comprising said chimeric gene, to a virus comprising a nucleic acid sequence encoding a putative soybean isoflavone-O-methyltransferase and to an expression cassette comprising said nucleic acid sequence operably linked to a promoter. Finally, the application refers to methods of selecting an isolated polynucleotide that affects the expression of a flavonoid biosynthetic enzyme, methods of obtaining a nucleic acid fragment encoding a flavonoid biosynthetic enzyme polypeptide and methods for positive selection of a cell transformed by the above mentioned chimeric gene or the above mentioned expression cassette.

2. **Reference is made to the following documents :**

   D1: HE XIAN-ZHI ET AL: 'Stress responses in alfalfa (Medicago sativa L). XXII. cDNA cloning and characterization of an elicitor-inducible isoflavone 7-0-methyltransferase.' PLANT MOLECULAR BIOLOGY JAN. 1, 1998, vol. 36, no. 1, 1 January 1998 (1998-01-01), pages 43-54, -& HE, X.Z., ET AL.:

'Medicago sativa 7-O-methyltransferase (7- IOMT(6)) mRNA, complete cds.' EMBL ACCESSION NO:AF000975, 5 November 1997 (1997-11-05)-& HE X.Z., ET AL.: 'Medicago sativa 7-O- methyltransferase (7-IOMT(9)) mRNA, complete cds.' EMBL ACCESSION NO:AF000976, 5 November 1997 (1997-11-05)-& HE X.Z., ET AL.: 'Medicago sativa isoflavone-O-methyltransferase mRNA, complete cds.' EMBL ACCESSION NO:U97125, 5 November 1997 (1997-11-05).

D2: DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US1977 POULTON J E ET AL: 'O METHYLATION OF FLAVONOID SUBSTRATES BY A PARTIALLY PURIFIED ENZYME FROM SOYBEAN CELL SUSPENSION CULTURES' Database accession no. PREV197764047255 & ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS 1977, vol. 180, no. 2, 1977, pages 543-549.

3. Novelty; article 33(2) PCT.

3.1 Due to the clarity problem mentioned in point VIII-2 of the present International Preliminary Examination Report (IPER), the International Preliminary Examination Authority (IPEA) considers that every soybean cell fit the definition of claims 7 and 8. Thus, claims 7 and 8 can not be considered as new.

Due to the clarity problem mentioned in point VIII-3 of the present IPER, the IPEA considers that every polynucleotide fit the definition of claim 18. Thus the subject-matter of claim 18 can not be considered as new (article 33(2) PCT).

3.2 The subject-matter of claims 1-6, 9-17 and 19-23 (specific sequence of a soybean 7-O-methyltransferase) has never been disclosed in the documents cited in the International Search Report (ISR). Therefore, claims 1-6, 9-17 and 19-23 are considered as new in the sense of article 33(2) PCT.

4 Lack of inventive step; article 33(3) PCT.

The most relevant document for the evaluation of the inventiveness of the claims is the document D1.
D1 discloses the cDNA cloning and characterization of an elicitor-inducible isoflavone 7-O-methyltransferase (p. 43, Title and Abstract, lines 5-6 and p. 47, Fig. 2). D1 states that the isoflavone-O-methyl transferase (IOMT) was expressed in Escherichia coli and that the recombinant enzyme was functional (p. 43, Abstract, lines 8-11; p. 46, Expression of alfalfa IOMT in E.coli and enzyme activity assay; p.49, Substrate and products specificity of IOMT expressed in E.coli and p. 49, Table 1). In order to express the IOMT in E. coli, the cDNA was cloned in the expression vector pET15b, a vector comprising regulatory sequences suitable for expression in E. coli. Moreover, D1 states that an alfalfa IOMT probe also hybridized to genomic DNA fragments from chickpea, **soybean**, bean and cowpea under low-stringency conditions (p. 50, Presence and genomic organization of IOMT in alfalfa and other species, lines 8-12), suggesting that a gene sharing similarities with the alfalfa IOMT gene could exist in soybean.

In view of the teaching of D1, the problem to be solved by the present application is the provision of further nucleic acid and protein sequences encoding an isoflavone-O-methyltransferase.

The application solves the problem by the provision of nucleic acid and protein sequences of soybean isoflavone-O-methyltransferases.

Knowing from D1 the importance of the isoflavonoid metabolism in the synthesis of phytoalexins like the medicarpin and knowing the nucleic acid and protein sequences of a enzyme involved in said metabolism, the skilled person would have contemplated isolating nucleic acid sequences encoding this enzyme in other plants and especially economically important plants like soybean using technics well-known in the art.

Moreover, the fact that an alfalfa IOMT probe is able to hybridize with soybean genomic DNA fragments would have given him good expectation of success in isolating such sequences.

Therefore, claims 1-4, 10 and 16-18 can not be considered as inventive in the sense of article 33(3) PCT.

The use of a non-inventive nucleic acid for the generation of a chimeric gene, the transformation of a host cell or the introduction in a virus using well-known technics can not be considered as inventive. Therefore, claims 5-9 and 19 lack inventive step (article 33(3) PCT).

The use of a non-inventive polynucleotide in well-known methods can also not be considered as inventive. Therefore, claims 11-15 and 20-23 can not be considered as inventive in the sense of article 33(3) PCT.

Re Item VIII

Certain observations on the international application

1. Claim 5 of the present application refers to a chimeric gene. In this claim, it is not mentioned if the regulatory sequences could be the "natural" regulatory sequences of the gene or not. The attention of the applicant is drawn to the fact that, even if the definition of a chimeric gene is given in the description, said definition should also appear in the wording of the claim since, according to the PCT Gazette of the 29.10.98 "PCT International Preliminary Examination Guidelines", Chapter III-4.2 : a claim should be drafted in such way that its "meaning is clear from the wording of the claim alone".

2. Claim 7 refers to a host cell comprising an isolated polynucleotide of claim 1. The attention of the applicant is drawn to the fact that the wording of this claim encompasses all the soybean cells expressing naturally an isoflavone-O-methyltransferase. These cells are well-known from the skilled person and would therefore deprive claim 7 of novelty.
   This remark is also valid for claim 8.

3. Claim 11 lacks clarity for the following reasons :
   (i) **Every** nucleotide sequence will comprise **at least one of 30** contiguous nucleotides derived from an isolated polynucleotide of claim 1.
   This remark also applies to claims 14, 15 and 18.

(ii) The wording "a nucleotide sequence of at least [one] 30 contiguous nucleotides **derived** from an isolated polynucleotide of claim 1" is unclear. Does it mean that the sequence **consists** of 30 contiguous nucleotides from an isolated polynucleotide of claim 1 or does this wording include sequences having different mutations like substitution, deletions, etc... of said sequence of at least 30 nucleotides ? In the latter case, the putative mutation should be precisely characterized.
This remark is also valid for claims 14, 15 and 18.

(iii) Since the polynucleotide of claim 1 is not limited to a sequence encoding a polypeptide of at least 348 amino acids that has at least 80 % identity (...) but can also **comprise** any other additional sequence, the polynucleotide of claim 11(a) which should comprise "at least [one of] 30 contiguous nucleotides from an isolated polynucleotide of claim 1" can also include polynucleotides which are totally unrelated with the present application.
This remark is also valid for the polynucleotide of claim 18.

(iv) In claim 11 (d), it is not clear the level of which polypeptide should be compared between plant cell containing the isolated polynucleotide and plant cell that does not contain the isolated polynucleotide.
This remark also applies to independent claim 13.

4. Claim 13 refers to a method of selecting an isolated polynucleotide that affects the level of expression of a flavonoid biosynthetic enzyme polypeptide in a plant cell. The IPEA considers that the method disclosed in claim 13 will not allow the selection of polynucleotide that affects the level of expression of a flavonoid biosynthetic enzyme but is rather a method of transformation of a plant cell with a nucleic acid encoding a flavonoid biosynthetic enzyme.

5. Claim 20 refers to a method for positive selection of a transformed cell. This claim lacks clarity since nothing is said about how to select the cells which allow expression of the polynucleotide in an amount sufficient to alter isoflavone levels in the cell.

6. As a general remark, the function given for the polypeptides of the present application is purely speculative and based on sequence similarities. The attention of the applicant is drawn to the fact that there are no concrete evidences that the

polypeptides of the present application are really implied in the biosynthesis of flavonoids. Therefore, all the claims relating to the function of said polypeptides in the flavonoids biosynthesis could be considered as lacking of support in the description.

Moreover, the attention of the applicant is drawn to the fact that, as a general rule, the IPEA considers that no inventive step can be recognized for a polypeptide having no proven function.